Published in final edited form as:

Pharmacol Ther. 2010 April ; 126(1): 56–68. doi:10.1016/j.pharmthera.2010.01.002.

Chemokines, neuronal-glial interactions, and central processing of neuropathic pain

Yong-Jing Gao and Ru-Rong Ji

Pain Research Center, Department of Anesthesiology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115

Abstract

Millions of people worldwide suffer from neuropathic pain as a result of damage to or dysfunction of the nervous system under various disease conditions. Development of effective therapeutic strategies requires a better understanding of molecular and cellular mechanisms underlying the pathogenesis of neuropathic pain. It has been increasingly recognized that spinal cord glial cells such as microglia and astrocytes play a critical role in the induction and maintenance of neuropathic pain by releasing powerful neuromodulators such as proinflammatory cytokines and chemokines. Recent evidence reveals chemokines as new players in pain control. In this article, we review evidence for chemokine modulation of pain via neuronal-glial interactions by focusing on the central role of two chemokines, CX3CL1 (fractalkine) and CCL2 (MCP-1), because they differentially regulate neuronal-glial interactions. Release of CX3CL1 from neurons is ideal to mediate neuronal-to-microglial signaling, since the sole receptor of this chemokine, CX3CR1, is expressed in spinal microglia and activation of the receptor leads to phosphorylation of p38 MAP kinase in microglia. Although CCL2 was implicated in neuronal-to-microglial signaling, a recent study shows a novel role of CCL2 in astroglial-to-neuronal signaling after nerve injury. In particular, CCL2 rapidly induces central sensitization by increasing the activity of NMDA receptors in dorsal horn neurons. Insights into the role of chemokines in neuronal-glial interactions after nerve injury will identify new targets for therapeutic intervention of neuropathic pain.

Keywords

CCL2/MCP-1; CX3CL1/fractalkine; astrocytes; microglia; nerve injury; spinal cord

1. Introduction

Neuropathic pain is a common reason for a clinic visit. It can be associated with many types of injuries to the nervous system, such as trauma, nerve compression, inflammation, and infection or be a consequence of neurodegenerative diseases (e.g. diabetes, multiple sclerosis), tumor infiltration, surgeries, as well as side effects of drug treatment (e.g. chemotherapy, antiretroviral therapy) (Dworkin et al., 2003; Ji & Strichartz, 2004; Kehlet et al., 2006; Woolf & Mannion, 1999). Neuropathic pain can manifest as spontaneous pain, allodynia (pain evoked by a normally innocuous stimulus), and hyperalgesia (enhanced pain evoked by a noxious stimulus). Particularly, tactile allodynia is a cardinal symptom of neuropathic pain (Dworkin et al., 2003). Neuropathic pain can persist for months and years, even after the primary tissue

Correspondence: Ru-Rong Ji (rrji@zeus.bwh.harvard.edu) and Yong-Jing Gao (yjing@zeus.bwh.harvard.edu), Department of Anesthesiology, Brigham and Women's Hospital, 75 Francis Street, Medical Research Building, Room 604, Boston, MA 02115 Tel: (617) 732-8852; Fax: (617) 730-2801.

damage has healed. Current treatments have only produced limited relief of this pain in a portion of patients (Costigan et al., 2009b; Dworkin et al., 2003).

Based on the association of clinical neuropathic pain with the nervous system injuries, several animal models have been used to investigate the neuropathic pain mechanisms and test novel analgesics, in which the sciatic nerve, spinal nerve, or DRG is intentionally damaged. These models include transection of the sciatic nerve (Devor & Wall, 1981), chronic constriction of sciatic nerve (CCI) (Bennett & Xie, 1988), partial sciatic nerve ligation (PSNL) (Seltzer et al., 1990), spinal nerve ligation (SNL) (Kim & Chung, 1992), spared nerve injury (SNI) (Decosterd & Woolf, 2000), and chronic compression of the DRG (CCD) (Hu & Xing, 1998). Neuropathic pain was also induced by infection, inflammation, or demyelination of the sciatic nerve (Wallace et al., 2003), as well as by chemotherapy (e.g., Paclitaxel) (Polomano et al., 2001) and toxin [(e.g., 2'-3'-dideoxycytidine (ddC)] (Joseph et al., 2004).

It is generally believed that neuropathic pain is an expression of neural plasticity, which can occur as both peripheral sensitization, an increase in the sensitivity and excitability of primary sensory neurons in the peripheral nervous system (PNS), and central sensitization, an increase in the activity and excitability of nociceptive neurons in the spinal cord and brain in the central nervous system (CNS), and lead to the development and maintenance of neuropathic pain (Ji et al., 2003; Julius & Basbaum, 2001; Woolf & Salter, 2000). After nerve injury, inflammatory mediators such as proinflammatory cytokines, chemokines, prostaglandins, histamine, serotonin, bradykinin, and nerve growth factors are released from injured nerve fibers and adjacent immune cells (Abbadie, 2005; Ji & Strichartz, 2004; Sommer & Kress, 2004). These mediators can directly act on DRG neuronal cell bodies and axons leading to peripheral sensitization (Kawasaki et al., 2008b; Schafer et al., 2003; Scholz & Woolf, 2002; Sommer & Kress, 2004). Nerve injury-induced central sensitization can manifest as an increase in glutamate NMDA and AMPA receptors-mediated excitatory synaptic transmission in dorsal horn neurons. It can also manifest as a decrease in GABA and glycine receptor-mediated decrease or loss of inhibitory synaptic transmission (disinhibition) (Coull et al., 2005; Ji et al., 2003; Woolf & Mannion, 1999). In addition, an increase in descending facilitation also contributes to central sensitization after nerve injury (Porreca et al., 2002).

In recent years, it is increasingly recognized that non-neuronal cells such as immune cells (macrophages, lymphocytes) and glial cells in the PNS (e.g., Schwann cells and satellite cells) and CNS (e.g., astrocytes and microglia) also play a critical role in chronic pain processing (Ji et al., 2006; McMahon & Malcangio, 2009; Milligan et al., 2008; Milligan & Watkins, 2009; Romero-Sandoval et al., 2008; Scholz & Woolf, 2007). Nerve injury induces substantial changes in both microglia and astrocytes in the spinal cord (DeLeo et al., 2004; Jin et al., 2003; Zhuang et al., 2006). Inhibition of microglial activation by minocycline prevents/delays neuropathic pain development (Hua et al., 2005; Ledebor et al., 2005; Raghavendra et al., 2003). Intrathecal injection of astroglial toxin fluorocitrate (Hosoi et al., 2004; Milligan et al., 2003) and L-alpha-aminoadipate (Zhuang et al., 2006) also reverses nerve injury- or nerve inflammation-induced mechanical allodynia. Further, inhibition of microglial signaling by inhibiting the action of P2X4 and p38 MAPK or activating cannabinoid receptor type-2 or inhibition of astroglial signaling by inhibiting c-Jun-N-terminal kinase (JNK) or matrix metalloprotease-2 also attenuates neuropathic pain (Jin et al., 2003; Kawasaki et al., 2008a; Romero-Sandoval et al., 2009; Tsuda et al., 2003; 2004; Zhuang et al., 2006). These data support an important role of spinal microglia and astrocytes in enhancing neuropathic pain.

It is generally believed that spinal glial cells enhance and maintain neuropathic pain by releasing potent neuromodulators, such as proinflammatory cytokines and chemokines and growth factors (Abbadie, 2005; Abbadie et al., 2009; Gao et al., 2009; Inoue, 2006; Milligan & Watkins, 2009; Trang et al., 2009; Watkins & Maier, 2002; White & Wilson, 2008). While

the role of proinflammatory cytokines (e.g. TNF- α , IL-1 β , and IL-6) in neuropathic pain sensitization has been well demonstrated (Arruda et al., 1998; Ledeboer et al., 2005; Lee et al., 2004; Milligan et al., 2003; Moalem & Tracey, 2006; Ohtori et al., 2004) and the mechanisms of these cytokines in central sensitization have also been explored (Guo et al., 2007; Kawasaki et al., 2008b), the role of chemokines in neuropathic pain is far from clear. Chemokines are small proteins that were initially characterized as chemotactic peptides controlling the trafficking of leukocytes (Charo & Ransohoff, 2006). Increasing evidence suggests that chemokines are involved in neuroinflammation at different anatomical locations, including injured nerve, dorsal root ganglion (DRG), spinal cord, and brain (Mennicken et al., 1999; Miller et al., 2008; Scholz & Woolf, 2007; White et al., 2007) and contribute to chronic pain processing (Abbadie et al., 2009). In this article, we review the evidence for the involvement of chemokines and chemokine receptors in neuropathic pain, with emphasis on their central role in regulating neuronal-glial interactions and neural plasticity.

2. Chemokines, chemokine receptors, and neurodegeneration

2.1. Chemokines

Chemokines are a family of functionally related small secreted molecules (8–14 kD) named “chemo-kine” because of leukocyte chemoattractant and cytokine-like activities (Asensio & Campbell, 1999). This family is composed of about 50 related molecules in humans, with close homologues in other mammalian species (Charo & Ransohoff, 2006). Each chemokine contains 70–100 amino acids, with 20–95% sequence identity to others including conserved cysteine residues (Bonecchi et al., 2009). According to cysteine's number and spacing, four chemokine subfamilies have been defined: CC, CXC, XC, and CX3C subfamilies (Bajetto et al., 2002; Laing & Secombes, 2004; Luster, 1998). The CC chemokines form the largest subfamily that is characterized by the adjacent positions of the first two of total four cysteine residues. The CC family has 28 members with a large spectrum of actions and attracts monocytes, eosinophils, basophils, T lymphocytes, natural killer (NK) cells, and dendritic cells (Savarin-Vuillat & Ransohoff, 2007; Ubogu et al., 2006).

The CXC chemokines is the second largest group that is characterized by the intervention of a single amino acid between the first two cysteine residues. Depending on the presence or absence of the sequence motif glutamic acid–leucine–arginine (ELR) near the N-terminus, the CXC subfamily can be divided into two groups: ELR-positive and ELR-negative, displaying different functions. The CXC chemokines with ELR motif bind and activate CXCR2, specifically acting on neutrophils and other CXCR2-positive cells, but those without the ELR motif act primarily on lymphocytes and monocytes (Savarin-Vuillat & Ransohoff, 2007; Ubogu et al., 2006).

The C chemokine family includes two molecules with only two cysteine residues. They can act on lymphocytes, but not on neutrophils or monocytes. The CX3C family has only one member with three intervening amino acids in-between the first two cysteine residues. CX3CL1 (fractalkine) can be soluble as well as membrane-bound and acts as an adhesion molecule or a chemoattractant for T cells and NK cells (Savarin-Vuillat & Ransohoff, 2007; Ubogu et al., 2006).

Chemokines were initially named by its biological function. Since 2000, a new chemokine classification system has been used, in which chemokines are considered as chemokine ligands (L) (Zlotnik & Yoshie, 2000). Therefore, each chemokine has a designation of CCL, CXCL, XCL, or CX3CL. Most chemokines have two names, one reflecting a particular biological aspect, such as monocyte chemoattractant protein-1, (MCP-1) and another reflecting its structure, such as CCL2 (Bajetto et al., 2002). In this review, we choose to use structural names of chemokines to better match with corresponding chemokine receptors.

In the CNS, different types of cells have been identified as sources of chemokines, including microglia, astrocytes, neurons and endothelial cells (Bajetto et al., 2002; Cartier et al., 2005; Mennicken et al., 1999). Except for CX3CL1 (fractalkine) and CXCL12, which are principally expressed by neurons and astrocytes, respectively (Bajetto et al., 1999; Harrison et al., 1998), most chemokines are not constitutively expressed but can be induced during diverse neurodegenerative disease conditions (Bajetto et al., 2002; Cartier et al., 2005).

2.2. Chemokine receptors

All chemokines exert their functions by activating surface receptors that are seven-transmembrane-domain G-protein-coupled receptors (GPCRs). To date, 19 chemokine receptors have been cloned. The nomenclature of these receptors is CC, CXC, XC, or CX3C followed by R and then a number. The chemokine receptors include ten CC receptors (from CCR1 to CCR10), seven CXC receptors (CXCR1–7), one XCR1 and one CX3CR1. Except for some monogamous chemokine–chemokine receptor pairs, such as CXCL13–CXCR5, CXCL16–CXCR6, and CX3CL1–CX3CR1, most chemokines activate multiple receptors. Vice versa, a single receptor can be activated by diverse chemokines (Charo & Ransohoff, 2006).

The structure of chemokine receptors is a single polypeptide chains spanning 7 times the membrane, with an acidic N terminal extracellular domain and a serine/threonine-rich intracellular C-terminal domain. Two disulfide bonds in-between the N-terminal domain and the second extracellular loop and the first and third extracellular loops are normally required for the definition of the molecular structure (Bonecchi et al., 2009). Chemokine receptors activate various signaling pathways, such as the mitogen-activated protein kinase (MAPK) pathway, the phospholipase C (PLC) pathway, and the phosphatidylinositol-3 kinase (PI3K) pathway (Bajetto et al., 2002; Cartier et al., 2005), leading to varied functional outcomes, including adhesion, polarization, and chemotaxis.

In vitro and *in vivo* studies have shown that many chemokine receptors, including the majority of the CCR family (CCR1 to CCR6), all the members of the CXCR family, and CX3CR1 are expressed in the CNS (Cartier et al., 2005; Mines et al., 2007). These chemokine receptors are expressed by astrocytes, neurons and microglia (Cartier et al., 2005; Mennicken et al., 1999; Mines et al., 2007). While some chemokines receptors, such as CCR1, CCR2, CCR3, CCR5, CXCR2, CXCR3, CXCR4 and CX3CR1 are constitutively expressed in the CNS, the majority of chemokines are detected under pathological conditions (Cartier et al., 2005).

2.3. Chemokines in neurodegenerative diseases

In addition to well-established role in the immune system, chemokines are also involved in several other processes throughout the body, including cardiogenesis, vascular development, cell proliferation, angiogenesis, and metastasis (Bonecchi et al., 2009; Rossi & Zlotnik, 2000). In particular, chemokines play an important role in the CNS under both physiological and pathological conditions (Ambrosini & Aloisi, 2004; Bajetto et al., 2002; Cartier et al., 2005). In physiological conditions, CXCL1, CXCL8, and CXCL12 regulate neurotransmitter release and modulate ion channel activity at both presynaptic and postsynaptic sites (Bertollini et al., 2006). CXCL12–CXCR4 and CXCL1–CXCR2 regulate CNS development (Giovannelli et al., 1998; Limatola et al., 2000). Chemokines and their receptors are especially involved in the pathogenesis of neurodegenerative diseases such as multiple sclerosis (MS), Alzheimer's disease (AD), as well as in neurological disorders, such as stroke and trauma (Mennicken et al., 1999; Savarin-Vuillat & Ransohoff, 2007; Ubogu et al., 2006).

MS is a chronic inflammatory disease, which can manifest as experimental autoimmune encephalomyelitis (EAE) in animals. EAE is a CD4(+) T lymphocyte-mediated CNS disease

characterized by mononuclear cell infiltration, demyelination, and paralysis (Murphy et al., 2002). The interaction of chemokines and their receptors plays a critical role in infiltration of inflammatory cells into the spinal cord and brain (Ubogu et al., 2006). Following CNS inflammation, microglia and astrocytes become reactive and increase the expression of chemokines and chemokine receptors (Ambrosini & Aloisi, 2004). For example, a number of chemokines such as CCL2-5, CCL7, CCL8, CXCL1, CXCL10, and CXCL12 are found in the brain of MS patients (Calderon et al., 2006; McManus et al., 1998; Simpson et al., 1998; 2000b; Van Der Voorn et al., 1999). CCL2, CCL7 and CCL8 are expressed on hypertrophic astrocytes and inflammatory cells within the lesion center (McManus et al., 1998; Van Der Voorn et al., 1999). CXCL1 is found in activated microglia localized on the border of MS lesions (Filipovic et al., 2003). In addition, several chemokine receptors, including CCR1, CCR2, CCR3, CCR5, CCR8, CXCR2, and CXCR3 are also found in post-mortem CNS tissue of MS patients (Balashov et al., 1999; Filipovic et al., 2003; Simpson et al., 2000a; Trebst et al., 2003). CCR1, CCR2, CCR3 and CCR5 are expressed in macrophages/microglia in areas of severe inflammation and necrosis of MS (Balashov et al., 1999; Simpson et al., 2000a). CCR2 and CCR5 are present on infiltrating lymphocytes; and CCR3 and CCR5 are also expressed in astrocytes (Simpson et al., 2000a). It appears that chemokine receptors are constitutively expressed in neurons but induced in immune cells and glial cells in the CNS after injury.

Chemokines directly contribute to neurodegeneration and disease progression in the EAE model. For example, CCR2 knockout mice fail to develop clinical EAE or CNS histopathology and demonstrate a significant reduction in T cell- and CNS-infiltrating monocyte populations. Compared with control mice, peripheral lymphocytes from CCR2 knockout mice produce comparable levels of interferon-gamma (IFN-gamma) and interleukin (IL)-2 in response to antigen-specific re-stimulation (Fife et al., 2000). Behavioral studies show that the severity of EAE is also reduced in CCR1 knockout mice (Rottman et al., 2000). Further, CCR8 deficiency significantly slows down the progression of EAE (Murphy et al., 2002).

A recent study shows that a single chemokine-receptor interaction of CCL19 and CCR7 can serve as a CNS leukemia entry signal. T-cell acute lymphoblastic leukemia is a blood malignancy afflicting mainly children and adolescents. These leukemia patients are at an increased risk of CNS relapse. CCR7 is an essential adhesion signal required for the targeting of leukemic T-cells into the CNS (Buonamici et al., 2009). Silencing of either CCR7 or its chemokine ligand CCL19 in an animal model of leukemia inhibits CNS infiltration of T-cells (Buonamici et al., 2009).

AD is characterized by progressive loss of neurons, leading to deficits in thought, learning, and memory. Although the etiology of AD remains unclear, glial cell-mediated neuroinflammation plays an important role in its pathogenesis (Tuppo & Arias, 2005). A number of chemokines and their receptors are expressed or up-regulated in brain tissues from AD patients. These include chemokines CCL2, CCL3, CCL4, CCL5, CCL8, CXCL8, and CXCL10 and chemokine receptors CXCR2, CXCR3, CCR3, CCR5, and CXCR4 (Horuk et al., 1997; Weeraratna et al., 2007; Xia et al., 1997; Xia et al., 2000; Xia et al., 1998). CXCL8, produced by microglia and astrocytes, is the most inducible chemokine in the brain of AD patients (Tuppo & Arias, 2005). Of interest is CXCR2, the receptor of CXCL8, strongly up-regulated on dystrophic neurites located in senile plaques (Xia & Hyman, 2002). Another example is for CCL3 and its receptor CCR5. While CCL3 is primarily found in neurons, CCR5 is increased in reactive microglia associated with amyloid deposits (Xia et al., 1998). Additionally, CXCR3 and its ligand CXCL10 are expressed in neurons and astrocytes, respectively, in AD brain (Xia et al., 2000). The distinct up-regulation of chemokines and chemokine receptors in different cell types suggest a role of these receptors in AD pathology via neuronal-glial interactions.

3. Chemokines and neuropathic pain

The expression, distribution and the function of chemokines and chemokine receptors in neuropathic pain have been investigated in different animal models of neuropathic pain, as described above. Although several chemokines/chemokine receptor pairs have been implicated in neuropathic pain, the CX3CL1/CX3CR1 and CCL2/CCR2 are two of the best studied pairs for neuropathic pain, which will be discussed below in great detail.

In addition to CCL2 and CX3CL1, other chemokines are also involved in pain regulation. Oh et al (2001) demonstrated that the chemokine CXCL12 (SDF-1, stromal cell-derived factor-1), CCL5, or CCL3 produce pain hypersensitivity by directly exciting primary nociceptive neurons. CXCL12/CXCR4 have been implicated in neuropathic pain sensitization after HIV1-associated peripheral neuropathy (Bhangoo et al., 2007b; Bhangoo et al., 2009). In particular, Zhao et al. (2007) reported that CCL21 could modulate thalamic nociceptive processing after spinal cord injury through remote activation of thalamic microglia. Electrical stimulation of the spinothalamic tract induces an increase in thalamic CCL21 levels. Further, injection of CCL21 into the thalamus transiently activates microglia and induces pain-related behaviors (Zhao et al., 2007). Thus CCL21 may serve as another neuronal-microglial signaling molecular in central neuropathic pain.

3.1 CX3CL1/CX3CR1 and neuropathic pain

3.1.1. CX3CL1/CX3CR1 distribution and regulation after nerve injury—CX3CL1 is the only member of CX3CL subfamily and characterized by two distinct forms: a membrane-bound form that displays adhesion properties and a soluble form that is cleaved from the cell membrane and has chemotactic properties (Bazan et al., 1997). Two distinct forms of CX3CL1 can exist with intrinsically different spatial properties and functions (Bazan et al., 1997; Pan et al., 1997).

In the nervous system, the full length CX3CL1 is constitutively expressed in spinal cord and DRG neurons (Lindia et al., 2005; Verge et al., 2004). Following nerve injury (CCI) or inflammation (SIN), the overall expression of CX3CL1 mRNA does not change in the DRG and spinal cord (Verge et al., 2004). On the contrary, SNL induces a marked reduction of the membrane-bound CX3CL1 in the DRG (Zhuang et al., 2007), suggesting a possible cleavage and release of this chemokine after nerve injury. CX3CL1 is also induced in spinal astrocytes by a modified SNL (Lindia et al., 2005).

CX3CR1, the only receptor of CX3CL1, is found in peri-neuronal glia in the DRG but predominantly in microglia in the spinal cord (Verge et al., 2004). Importantly, CX3CR1 expression in microglia is upregulated under conditions of neuropathic pain induced by SIN, CCI (Lindia et al., 2005; Verge et al., 2004), and SNI (Holmes et al., 2008). The most dramatic spinal upregulation of CX3CR1 and the most distinct localization of this receptor in spinal microglia was found after SNL (Zhuang et al., 2007) (Fig. 1).

3.1.2. CX3CL1/CX3CR1 and neuronal-microglial signaling in the spinal cord—

As we have discussed above, CX3CL1 is mainly expressed by neurons (Harrison et al., 1998) while CX3CR1 is primarily expressed by microglia in the spinal cord dorsal horn (Harrison et al., 1998; Lindia et al., 2005; Verge et al., 2004) (Fig. 1). This complimentary distribution pattern suggests a possible involvement of CX3CL1 in signaling between neurons and microglia (Harrison et al., 1998; Nishiyori et al., 1998).

In vitro studies show that excitotoxic stimulus (glutamate) of cultured cortical neurons triggers the cleavage of CX3CL1 from the membranes. In addition, the medium sampled at 3 hr post-glutamate treatment shows significant chemotaxis activity for microglia. This activity is largely

inhibited by a neutralizing antiserum against the CX3CR1 receptor (Chapman et al., 2000), suggesting a role of cleaved CX3CL1 in recruiting microglia.

Several proteases have been implicated in the cleavage of CX3CL1. For example, the lysosomal cysteine protease, cathepsin S (CatS) contributes to neuropathic pain development via CX3CL1 cleavage (Clark et al., 2007b; Clark et al., 2009a). Notably, incubation of cultured DRG neurons with CatS reduces CX3CL1 expression at the cell surface but increases CX3CL1 levels in the DRG culture media. Further, intrathecal injection of CatS induces mechanical allodynia in wild-type but not CX3CR1-knockout mice (Clark et al., 2007b). In a follow-up study, these authors demonstrated that in the dorsal horn of neuropathic animals, noxious-like electrical stimulation of primary afferent fibers increases the release of soluble CXCL1 via CatS (Clark et al., 2009a).

Metalloproteases also play an important role in active cleavage of CX3CL1. Chapman et al. (2000) showed that CX3CL1 is rapidly cleaved in cultured cortical neurons, in response to an excitotoxic stimulus (glutamate), and a general metalloprotease inhibitor batimastat, dose dependently inhibits this cleavage. We have recently demonstrated an important role of MMP-9 and MMP-2 in glial activation and neuropathic pain development. After nerve injury, MMP-9 is upregulated in DRG neurons and contributes to microglial activation and early-phase neuropathic pain development via active cleavage of IL-1 β in the early phase (Kawasaki et al., 2008a). In sharp contrast, MMP-2 is persistently up-regulated in spinal cord astrocytes after nerve injury and contributes to late-phase neuropathic pain development via active cleavage of IL-1 β in the late phase (Kawasaki et al., 2008a). Interestingly, a recent study showed that CX3CL1 is mainly processed by MMP-2 in liver cells (Bourd-Boittin et al., 2009). Therefore, it is attempting to postulate that MMP-9 and MMP-2 may also activate microglia in early- and late-phase of nerve injury, respectively, by active cleavage of CX3CL1.

In vivo studies further support the involvement of CX3CL1 in microglia activation. First, nerve injury induces CX3CR1 upregulation in spinal cord microglia (Lindia et al., 2005; Verge et al., 2004; Zhuang et al., 2007) (Fig. 1). Second, thermal hyperalgesia induced by intrathecal injection of CX3CL1 is blocked by minocycline (Milligan et al., 2005). Third, spinal administration of CX3CL1 activates p38 MAPK (Clark et al., 2007b; Zhuang et al., 2007), and the phosphorylated p38 (active p38) is predominantly expressed in spinal microglia in rats (Jin et al., 2003; Zhuang et al., 2007). Finally, intrathecal injection of a neutralizing antibody against CX3CR1 suppressed p38 activation. These results indicate that CX3CL1, after binding its sole receptor CX3CR1 and activating p38 MAPK, induces microglia activation (Fig. 2).

Activation of p38 MAPK induces synthesis of proinflammatory cytokines such as TNF- α , IL-1 β , and IL-6 (Ji & Suter, 2007). Recently, our electrophysiology study in superficial dorsal horn neurons shows that these proinflammatory cytokines, at low concentrations (1–10 ng/ml) induce central sensitization via distinct mechanisms. (1) TNF- α enhances excitatory synaptic transmission by increasing the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) and the amplitude of AMPA- or NMDA-induced currents. (2) IL-6 inhibits inhibitory synaptic transmission by reducing the frequency of spontaneous inhibitory postsynaptic currents (IPSCs) and the amplitude of GABA (gamma-amino butyric acid)- and glycine-induced currents. (3) Particularly, IL-1 β can both enhance excitatory synaptic transmission and reduce inhibitory synaptic transmission. These results suggest a powerful role of these proinflammatory cytokines in regulating synaptic plasticity and neuronal excitability (Kawasaki et al., 2008b). Trang et al. showed that in cultured microglia ATP activates p38 via P2X4 receptor, leading to the release of BDNF from microglia (Trang et al., 2009). BDNF was shown to induce central sensitization via disinhibition (Coull et al., 2005). After ATP stimulation, cultured microglia release BDNF, which produces a depolarizing shift in the anion reversal potential in spinal lamina I neurons, so that the polarity of currents activated by GABA

is reversed. This mechanism also occurs after peripheral nerve injury (Coull et al., 2005). Very recently, Clark et al. show that p38 also mediates IL-1 β release from spinal microglia after the activation TLR4, and this release requires P2X7 receptors (Clark et al., 2010).

3.1.3 CX3CL1/CX3CR1 and neuropathic pain behaviors—Behavioral studies show that CX3CL1 induces marked mechanical allodynia (Milligan et al., 2005; Milligan et al., 2004; Zhuang et al., 2007) and thermal hyperalgesia (Milligan et al., 2005; Milligan et al., 2004) in naive rats and mice (Clark et al., 2007b). CX3CL1-induced pain hypersensitivity is abrogated in CX3CR1 knockout mice (Clark et al., 2007b). In addition, a neutralizing antibody against CX3CR1 or CX3CL1 attenuates the development of mechanical allodynia in the CCI and SNL neuropathic pain models (Clark et al., 2007b; Milligan et al., 2005; Milligan et al., 2004; Zhuang et al., 2007). Finally, mechanical allodynia after PSNL does not develop in CX3CR1 knockout mice, whereas heat hyperalgesia still develops in these mice, suggesting that this receptor is particularly important for regulating mechanical allodynia (Clark et al., 2009b).

3.2 CCL2/CCR2 and neuropathic pain

CCL2, also known as monocytes chemoattractant protein 1 (MCP-1), can specifically recruit monocytes to sites of inflammation, infection, trauma, toxin exposure, and ischemia. Although CCL2 recognizes several receptors, including CCR1, CCR2, and CCR4 (Savarin-Vuaillet & Ransohoff, 2007; White et al., 2007), CCR2 is the preferred receptor (Jung et al., 2009; Kurihara & Bravo, 1996). In mouse tissues, CCR2 specifically binds CCL2 with 10 times higher affinity than CCL7 and MCP-3 and doesn't bind CCL3, CCL6 or CXCL1 (Kurihara & Bravo, 1996). By using transfected cells and bitransgenic reporter mice in which the CCR2 and CCL2 were labeled by different fluorescence, Jung et al (2009) also showed that CCL2 binds on CCR2, but not CXCR4, leading to endocytosis of the CCL2/CCR2 complex. A specific CCR2 antagonist, CCR2 RA, can completely block the appearance of CCL2/CCR2 endocytotic vesicles. Especially, increasing evidence has revealed an important role of CCL2/CCR2 signaling in the processing of neuropathic pain (Abbadie et al., 2009).

3.2.1. CCL2/CCR2 distribution and regulation after nerve injury—CCL2 expression in primary sensory neurons is well studied especially in conditions of nerve injury. Tanaka et al. (2004) reported a very rapid (< 4 h) upregulation of CCL2 in DRG neurons after PNL. Zhang and De Koninck (2006) showed that after nerve constriction CCL2 is induced in both small and large neurons that also express the transcription factor ATF-3, a marker for axonal injury, suggesting that CCL2 is increased mainly in injured neurons. However, Thacker et al. (2009) demonstrated that CCL2 is produced by both damaged (L5) and undamaged (L4) primary sensory neurons after ligation and transection of the L5 spinal nerve. CCL2 is also induced in DRG neurons in other neuropathic pain conditions, including sciatic nerve demyelination (Jung et al., 2008), chronic compression of the DRG (White et al., 2005b), axotomy, CCI and SNL (Jeon et al., 2009), as well as in an inflammatory pain condition after adjuvant injection (Jeon et al., 2008). While most studies show very low basal expression of CCL2 in DRG neurons (Jung et al., 2008; Tanaka et al., 2004; Zhang & De Koninck, 2006), Dansereau et al. showed that more than 40% small and medium DRG neurons express CCL2 in normal conditions (Dansereau et al., 2008). This discrepancy may result from differences in the sensitivity of antibodies and immunohistochemical procedures. Nevertheless, all the evidence suggests that CCL2 is highly inducible in the DRG after nerve injury. Double staining experiments indicate that CCL2 is co-expressed with the neuropeptides substance P and CGRP and the capsaicin receptor TRPV1 (Dansereau et al., 2008). It is of interest to notice that CCL2 is also induced in satellite cells surrounding neurons in the DRG, although this result is not specifically addressed by the authors (Jung et al., 2008). Thus MCP-1 can be induced both in neurons and satellite cells in the DRG.

CCR2 expression in the DRG is well documented too. CCR2 is partially colocalized with CCL2, suggesting a possible autocrine/paracrine role for CCL2/CCR2 signaling within the DRG (Jung et al., 2009). In situ hybridization shows that chronic constriction of DRG induces CCR2 mRNA expression in neurons and non-neuronal cells in both compressed (L4/L5) and adjacent non-compressed (L3/L6) DRGs (White et al., 2005b). CCR2 is also upregulated in the DRG after sciatic nerve demyelination (Jung et al., 2009; Jung et al., 2008; White et al., 2005b). Therefore, CCL2 can activate CCR2 in both neurons and glial cells in the DRG to modulate pain sensitivity.

Electrophysiological studies indicate that application of CCL2 increases $[Ca^{2+}]_i$ in cultured neonatal DRG neurons (Oh et al., 2001). DRG neurons isolated from animals exhibiting neuropathic pain behavior are strongly depolarized by CCL2 (Sun et al., 2006; White et al., 2005b). CCL2-induced sensitization of nociceptors may be caused by activation of TRP channels and inhibition of K^+ conductance (Jung et al., 2008; Sun et al., 2006; White et al., 2005a). Sun et al showed that CCL2 inhibits a voltage-dependent, non-inactivating outward current, presumably a delayed rectifier type K^+ conductance, which is known to regulate neuronal excitability (Yang et al., 2004). CCL2 in DRG neurons should be transported to peripheral terminals in the skin, as intradermal injection of MCP-1 directly induces mechanical hyperalgesia (Bogen et al., 2009).

Compared to well-characterized expression in the DRG, CCL2/CCR2 expression in the spinal cord is elusive and controversial. Previous studies showed CCL2 expression in primary afferents in the spinal cord (Dansereau et al., 2008; Zhang & De Koninck, 2006), whereas CCR2 is induced in spinal microglia, thus providing a great model for neuron-derived MCP-1 activation of spinal cord microglia after nerve injury (Thacker et al., 2009).

However, CCL2 in the spinal cord is not only expressed in primary afferents. CCL2 is colocalized with GFAP (Fig. 3), indicating an expression by astrocytes (Gao et al., 2009). In addition, CCL2 expression in astrocytes is increased after spinal nerve ligation (Gao et al., 2009) (Fig. 3) and spinal cord contusion injuries (Knerlich-Lukoschus et al., 2008). Accumulating evidence demonstrates that activated astrocytes *in vitro* produce CCL2 (Croitoru-Lamourey et al., 2003; El-Hage et al., 2005; Gao et al., 2009; Meeuwsen et al., 2003; Mojsilovic-Petrovic et al., 2007). CCL2 is also expressed in brain astrocytes after demyelinating lesions (Tanuma et al., 2006; Van Der Voorn et al., 1999), mechanical injury (Glabinski et al., 1996), entorhinodentate axon transaction (Babcock et al., 2003), and focal cerebral ischemia (Yan et al., 2007).

CCR2 expression in the spinal cord is also debated. An early immunohistochemical study showed CCR2 expression in spinal microglial cells (Abbadie et al., 2003). But recent studies also showed CCR2 expression in astrocytes (Knerlich-Lukoschus et al., 2008) and neurons (Gao et al., 2009). Reverse transcriptase (RT)-PCR reveals high level expression of CCR2 in the spleen but only low level expression of CCR2 in the DRG and spinal cord in naïve animals. Several lines of evidence demonstrate that CCR2 is constitutively expressed in spinal cord neurons (Gao et al., 2009). First, CCR2-GFP reporter mice (Jung et al., 2008) show a weak but clear GFP signal in dorsal horn neurons (Fig. 3). Second, *in situ* hybridization shows that CCR2 mRNA is undetectable in the spinal cord of naïve animals, which may result from lower sensitivity of *in situ* hybridization with digoxigenin labeling system compared with GFP reporter system. However, 3 days after spinal nerve ligation, CCR2 mRNA signal is clearly detected in deep dorsal horn neurons and motor neurons, indicating an upregulation of CCR2 mRNA after nerve injury (Gao et al., 2009). CCR2 upregulation is also found in spinal microglia after partial sciatic nerve injury (Abbadie et al., 2003) and in astrocytes after spinal cord contusion injuries (Knerlich-Lukoschus et al., 2008). Third and importantly, electrophysiological study showed that CCL2 can very rapidly (within minutes) increases

sEPSC and NMDA current in dorsal horn neurons (Fig. 4), suggesting the existence of functional CCR2 receptors in dorsal horn neurons (Gao et al., 2009). In contrast, application of CX3CL1 to spinal cord slices fails to increase sEPSC (Zhuang et al., 2007). Clearly, these data suggest that functional CCR2 and CX3CR1 are localized in different cells in the spinal cord.

3.2.2. CCL2/CCR2 and neuronal-microglial signaling in the spinal cord—CCL2 in DRG neurons can also be transported to central terminal in the spinal cord. Thus, CCL2 is found in SP- and CGRP-positive primary afferents in the superficial dorsal horn (Dansereau et al., 2008; Gao et al., 2009). Accumulation of CCL2 was observed distal to the dorsal root ligature after spinal nerve ligation, indicating an injury induced transport of CCL2 to central terminal (Thacker et al., 2009). Importantly, CCL2 is released in an activity dependent manner from the central terminals. An ex vivo study from isolated dorsal horn preparation with dorsal root attached showed that basal levels of CCL2 in spinal cord superfusates from sham and neuropathic animals were not significantly different. However, supramaximal electrical stimulation of the dorsal roots evoked rapid release of CCL2 from neuropathic, but not sham preparations (Thacker et al., 2009). These data suggest that following a peripheral nerve injury, CCL2 is released, in an activity dependent manner, from the primary afferents in the dorsal horn and may function as a neuromodulator.

Peripheral nerve injury not only induces upregulation of CCL2 and CCR2 (Abbadie et al., 2003; Gao et al., 2009; Jung et al., 2008; Zhang & De Koninck, 2006) but also activation of spinal microglia (Clark et al., 2007a; Ji & Suter, 2007; Jin et al., 2003; Watkins & Maier, 2003). The spatial profile of CCL2 expression in the spinal cord dorsal horn matches that of activated microglia (Beggs & Salter, 2007; Thacker et al., 2009; Zhang & De Koninck, 2006). Importantly, nerve injury induced-microgliosis in the spinal cord was prevented by spinal injection of CCL2 neutralizing antibody (Thacker et al., 2009; Zhang et al., 2007) or in mice lacking CCR2 (Zhang et al., 2007). Exogenous spinal administration of CCL2 induces microgliosis in wild-type but not in CCR2 knockout mice, although a high dose of CCL2 (3 injections, 2 µg per injection, over 6 days) is required (Zhang et al., 2007). Nerve injury-induced p38 activation in spinal microglia is also attenuated in CCR2 knockout mice (Abbadie et al., 2003; Zhang & De Koninck, 2006; Zhang et al., 2007). Recently, Thacker et al demonstrated that intraspinal CCL2 induced extensive microglial reaction in the ipsilateral dorsal horn (Thacker et al., 2009). Taken together, these data suggest that spinal CCL2/CCR2 signaling is critical for spinal microglial activation and the development of neuropathic pain after peripheral nerve damage (Fig. 2).

3.2.3. CCL2/CCR2 and astroglial-neuronal signaling in the spinal cord—As discussed above, CCL2 is also induced in spinal cord astrocytes after SNL. Strikingly, CCL2 is highly inducible in cultured astrocytes. A brief exposure of astrocytes to TNF- α induces >100 fold increase in CCL2 expression. TNF- α also produces a rapid and substantial increase in CCL2 release from astrocytes, in a JNK-dependent manner (Gao et al., 2009). Further, CCR2 is constitutively expressed in dorsal horn neurons (Gao et al., 2009; Gosselin et al., 2005). These data indicate CCL2 can also serve as a signaling molecule between astrocytes and neurons and contribute to central sensitization following nerve injury (Fig. 5).

CCL2 appears to have direct actions on spinal cord neurons. In isolated spinal cord slice preparation, application of CCL2 to spinal slices immediately increases the frequencies of sEPSCs in lamina II neurons of the dorsal horn (Gao et al., 2009), suggesting a presynaptic mechanism of CCL2 to enhance glutamate releases (Baba et al., 2003; Kohno et al., 2005). Furthermore, CCL2 increases the amplitudes of sEPSCs (Gao et al., 2009) suggesting a postsynaptic mechanism of CCL2 to enhance glutamate receptor function (Kohno et al., 2005). In support of postsynaptic mechanisms, CCL2 also rapidly (< 2 min) enhances NMDA-

and AMPA-induced inward currents in lamina II neurons (Gao et al., 2009) (Fig. 4a). Gosselin et al. demonstrate in neonatal cultures that CCL2 inhibits GABA-induced currents in spinal neurons without affecting the electrical properties of these neurons (Gosselin et al., 2005). Thus, CCL2 can further modulate inhibitory synaptic transmission in spinal cord neurons.

In parallel with electrophysiological evidence, behavioral evidence shows that spinal injection of CCL2 induces rapid heat hyperalgesia, starting at 15 min, peaking at 30 min, and recovering at 24 h (Gao et al., 2009). Moreover, incubation of spinal cord slice with CCL2 induces a rapid (within 5 minutes) phosphorylation of ERK (pERK) in superficial dorsal horn neurons (Fig. 4b) (Gao et al., 2009). ERK activation in dorsal horn neurons is nociceptive-specific and contributes importantly to the induction of central sensitization (Ji et al., 1999; Karim et al., 2001). Thus, pERK induction in dorsal horn neurons can serve as a marker for central sensitization (Gao & Ji, 2009). Once again, a rapid activation of ERK in dorsal horn neurons by CCL2 supports a direct action of CCL2 on spinal cord neurons and its involvement in central sensitization. ERK activation in dorsal horn neurons could induce central sensitization by activating NMDA receptors (Fig. 5).

Collectively, these data reveal a new form of neuronal-glial interaction. Apart from activating microglia via transcription-dependent microgliosis, CCL2 also has direct, rapid, and non-genomic actions on neurons. It can induce central sensitization, within minutes, by inducing ERK activation and enhancing excitatory synaptic transmission in dorsal horn neurons (Fig. 5).

3.2.4. CCL2/CCR2 and neuropathic pain behaviors—Several lines of evidence support a role of CCL2/CCR2 in promoting neuropathic pain. First, mice lacking CCR2 display a substantial reduction in mechanical allodynia after partial ligation of the sciatic nerve (Abbadie et al., 2003; Zhang et al., 2007). Second, intrathecal injection of CCR2 antagonist reversed tactile allodynia induced by focal peripheral nerve axon demyelination (Bhangoo et al., 2007a) or perineural gp120/hCD4 injury (Bhangoo et al., 2009). Third, mice over-expressing CCL2 in astrocytes exhibit enhanced pain sensitivity (Menetski et al., 2007). Fourth, intrathecal administration of CCL2 enhances pain hypersensitivity (Thacker et al., 2009). Finally, CCL2 neutralizing antibody reduces mechanical allodynia induced by SNL (Gao et al., 2009) or CCI (Thacker et al., 2009).

4. Therapeutic potentials and conclusions

Neural plasticity in both the PNS and the CNS contributes to the development and maintenance of neuropathic pain (Ji et al., 2003; Julius & Basbaum, 2001; Woolf & Salter, 2000). Thus, development of neuropathic pain therapeutics has been focusing on neuronal targets, in particular on blocking neurotransmission. Although some drugs, such as N-methyl-D-aspartic acid (NMDA) receptor antagonists, selective serotonin/norepinephrine reuptake inhibitors, opioid analgesics, sodium channel blockers, and tricyclic antidepressants have shown some effects in some patients (Dworkin et al., 2003), they often produce transient pain relief, because they only treat the pain symptoms but not the cause underlying disease progression such as neuroinflammation (Ji et al., 2009b). Furthermore, the side effects of these drugs, often CNS-related, such as nausea, sedation, drowsiness, dizziness, as well as development of analgesic tolerance and addiction after opioid treatment, have greatly limited their universal use (Dworkin et al., 2003).

Given the failure of current neuron-targeting strategies and important role of glial cells in the pathogenesis of neuropathic pain, targeting dysfunctional glial cells and neuronal-glial interactions have becoming attractive new strategies for the treatment of neuropathic pain. In animal studies, there is an increasing list of glia-modifying drugs that have demonstrated great

efficacy in reducing neuropathic pain-like behaviors. These potential glia-modifying drugs include microglial inhibitor (e.g., minocycline), cytokine inhibitors (e.g., IL-1 β antagonist anakinra, TNF- α inhibitor etanercept), ATP receptor antagonists (e.g., P2X4 and P2X7 antagonists), TLR antagonists (e.g., TLR2 and TLR4 antagonists), and cannabinoid CB2 receptor agonists, as well anti-inflammatory cytokines (e.g., IL-10) (reviewed in Inoue & Tsuda, 2009; Ji & Strichartz, 2004; Romero-Sandoval et al., 2008; Scholz & Woolf, 2007; Watkins & Maier, 2003).

Some glia-modifying drugs such as AV411 and propentofylline (SLC022) are currently being tested in clinical trials for neuropathic pain. AV411 is an orally bioavailable, centrally acting molecule. It was initially developed in Japan as a non-selective phosphodiesterase (PDE) inhibitor for treating bronchial asthma. But AV411 inhibits neuropathic pain in animal models via mechanisms that are independent on the PDE activity. Rather, AV411 acts as a glial cell regulator and suppresses the production of pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6), and may enhance the production of the anti-inflammatory cytokine IL-10. It also reduces signs of opioid tolerance and dependence. Daily systemic administration of AV411 for multiple days resulted in a sustained attenuation of CCI-induced allodynia (Ledeboer et al., 2007). Like AV411, propentofylline (SLC022) is also a PDE inhibitor and acts as an adenosine reuptake inhibitor too. It produces neuroprotective effects and has been studied as a possible treatment for Alzheimer's disease. Notably, propentofylline can effectively inhibit glia reactions, production of pro-inflammatory cytokines, and neuropathic pain in the spinal nerve transection model (Tawfik et al., 2007).

Given the important role of chemokines in neuropathic pain and neuronal-glia interactions, targeting chemokine signaling is emerging as an attractive new strategy for treating neuropathic pain. Direct inhibitors of chemokines or chemokines receptors have been developed, including monoclonal antibodies, antisense inhibitors, chemokines mutants, and small molecular antagonists (Mines et al., 2007). Particularly, pharmaceutical companies have been focusing on non-peptide small molecular antagonists against different chemokines receptors. Antagonists for more than 10 chemokine receptors such as CCR1-5, CCR8, CCR9, and CXCR1-4 have been developed. But most of them failed to show any indication of efficacy in clinical trials, in part due to the problems related to species selectivity, pharmacokinetic properties, and drug metabolism. Till now, only one small-molecule chemokine receptor antagonist, the anti-CCR5 molecule, maraviroc has won FDA approval for the use in combination with other antiretroviral agents in the treatment of HIV-1 infection. Nevertheless, there are still some chemokine receptor antagonists that are currently under clinical trials and may become promising therapeutics in the future (Pease & Horuk, 2009a; 2009b).

Chemokine receptor antagonists have also shown efficacy in animal models of neuropathic pain. For example, AMD3100, which blocks CXCL12 binding to CXCR4 and inhibits CXCL12-induced GTP-binding, calcium flux, and chemotaxis, also attenuates mechanical allodynia in the antiretroviral toxic neuropathy model (Bhangoo et al., 2007b). Intrathecal injection of CCR2 RA-[R], a CCR2 antagonist, reverses tactile allodynia induced by focal peripheral nerve axon demyelination (Bhangoo et al., 2007a) or perineural gp120/hCD4 injury (Bhangoo et al., 2009). Another CCR2 antagonist, PF-4136309, developed by Incyte (Phase II clinical trial), has been licensed to Pfizer for pain management (Pease & Horuk, 2009a).

A major reason for the failure of many chemokine receptor antagonists in clinical trials is their lack of efficacy. In addition to the problems related to species selectivity, pharmacokinetic properties, and drug metabolism, as described above, the lack of efficacy also results from redundancy in the chemokine network, since there are so many chemokines and one chemokine often has multiple receptors. Thus, it might be beneficial to simultaneously target several chemokine receptors. It will also be important to develop receptor antagonists that have CNS

permeability and actions, because chemokines in the CNS play a critical role in the development and maintenance of neuropathic pain, as we discussed.

In addition to direct targeting of chemokines and chemokine receptors, indirect targeting of chemokine synthesis and activation should also be considered for designing more successful therapeutics in the future (Fig. 6). NF- κ B and MAPK pathways are well known to control the synthesis of multiple chemokines. For example, both the JNK and ERK pathways are required for the synthesis of CCL2 in astrocytes, and JNK is also required for the release of CCL2 from astrocytes (Gao et al., 2009). Whereas p38 is activated in spinal microglia and may control the synthesis and release of chemokines in microglial cells (Costigan et al., 2009a; Ji & Suter, 2007). Importantly, inhibitors of JNK, p38, and MEK can effectively attenuate nerve injury-induced neuropathic pain in various animal models (reviewed in Ji et al., 2009a). As discussed above, several proteases, such as CatS, MMP-9, and MMP-2 contribute to the active cleavage of CX3CL1, and inhibitors of these proteases inhibit neuroinflammation and neuropathic pain in animal models (reviewed in Ji et al., 2009b; McMahon & Malcangio, 2009). Indeed, MAPK and MMP inhibitors can inhibit the synthesis, activation, or/and release of not only proinflammatory chemokines but also proinflammatory cytokines that are also critical for the pathogenesis of neuropathic pain.

In summary, management of neuropathic pain is a real clinical challenge. Increasing evidence suggests an important role of chemokines (e.g., CX3CL1 and CCL2) in the genesis of neuropathic pain via regulating neuronal-glial interactions. Thus targeting chemokine signaling may provide new therapeutics for the treatment of neuropathic pain. Although the pharmaceutical industry expends a lot of effort to generate potent chemokine receptor antagonists, most antagonists have failed in clinical trials due to the lack of efficacy. Given the redundancy in the chemokine system and a critical role of central chemokines for regulating neuronal-glial interactions, it might be beneficial to develop receptor antagonists that have CNS permeability and target several chemokine receptors. Indirect targeting of chemokine synthesis, release, and activation by kinase and protease inhibitors should also be considered to improve the efficacy in suppressing neuroinflammation and neuropathic pain.

Acknowledgments

This work was supported by NIH grants NS54932, NS67686, and DE17794.

Abbreviations

AD	Alzheimer's disease
AMPA	alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
CCI	chronic constriction injury
CNS	central nervous system
DRG	dorsal root ganglion
ERK	extracellular signal-regulated kinase
EPSC	excitatory postsynaptic currents
IL	interleukin
JNK	c-Jun N-terminal kinase
MAPK	mitogen activated protein kinase
MCP-1	monocytes chemoattractant protein-1

MS	multiple sclerosis
NMDA	N-methyl-D-aspartic acid
PNS	peripheral nervous system
SNL	spinal nerve ligation
TNF- α	tumor necrosis factor-alpha
TRPV1	transient receptor potential vanilloid type 1

References

- Abbadie C. Chemokines, chemokine receptors and pain. *Trends Immunol* 2005;26:529–534. [PubMed: 16099720]
- Abbadie C, Bhargoo S, De Koninck Y, Malcangio M, Melik-Parsadaniantz S, White FA. Chemokines and pain mechanisms. *Brain Res Rev* 2009;60:125–134. [PubMed: 19146875]
- Abbadie C, Lindia JA, Cumiskey AM, Peterson LB, Mudgett JS, Bayne EK, et al. Impaired neuropathic pain responses in mice lacking the chemokine receptor CCR2. *Proc Natl Acad Sci U S A* 2003;100:7947–7952. [PubMed: 12808141]
- Ambrosini E, Aloisi F. Chemokines and glial cells: a complex network in the central nervous system. *Neurochem Res* 2004;29:1017–1038. [PubMed: 15139300]
- Arruda JL, Colburn RW, Rickman AJ, Rutkowski MD, DeLeo JA. Increase of interleukin-6 mRNA in the spinal cord following peripheral nerve injury in the rat: potential role of IL-6 in neuropathic pain. *Brain Res Mol Brain Res* 1998;62:228–235. [PubMed: 9813345]
- Asensio VC, Campbell IL. Chemokines in the CNS: plurifunctional mediators in diverse states. *Trends Neurosci* 1999;22:504–512. [PubMed: 10529818]
- Baba H, Ji RR, Kohno T, Moore KA, Ataka T, Wakai A, et al. Removal of GABAergic inhibition facilitates polysynaptic A fiber-mediated excitatory transmission to the superficial spinal dorsal horn. *Mol Cell Neurosci* 2003;24:818–830. [PubMed: 14664828]
- Babcock AA, Kuziel WA, Rivest S, Owens T. Chemokine expression by glial cells directs leukocytes to sites of axonal injury in the CNS. *J Neurosci* 2003;23:7922–7930. [PubMed: 12944523]
- Bajetto A, Bonavia R, Barbero S, Piccioli P, Costa A, Florio T, et al. Glial and neuronal cells express functional chemokine receptor CXCR4 and its natural ligand stromal cell-derived factor 1. *J Neurochem* 1999;73:2348–2357. [PubMed: 10582593]
- Bajetto A, Bonavia R, Barbero S, Schettini G. Characterization of chemokines and their receptors in the central nervous system: physiopathological implications. *J Neurochem* 2002;82:1311–1329. [PubMed: 12354279]
- Balashov KE, Rottman JB, Weiner HL, Hancock WW. CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. *Proc Natl Acad Sci U S A* 1999;96:6873–6878. [PubMed: 10359806]
- Bazan JF, Bacon KB, Hardiman G, Wang W, Soo K, Rossi D, et al. A new class of membrane-bound chemokine with a CX3C motif. *Nature* 1997;385:640–644. [PubMed: 9024663]
- Beggs S, Salter MW. Stereological and somatotopic analysis of the spinal microglial response to peripheral nerve injury. *Brain Behav Immun* 2007;21:624–633. [PubMed: 17267172]
- Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 1988;33:87–107. [PubMed: 2837713]
- Bertollini C, Ragozzino D, Gross C, Limatola C, Eusebi F. Fractalkine/CX3CL1 depresses central synaptic transmission in mouse hippocampal slices. *Neuropharmacology* 2006;51:816–821. [PubMed: 16815480]
- Bhargoo S, Ren D, Miller RJ, Henry KJ, Lineswala J, Hamdouchi C, et al. Delayed functional expression of neuronal chemokine receptors following focal nerve demyelination in the rat: a mechanism for the development of chronic sensitization of peripheral nociceptors. *Mol Pain* 2007a;3:38. [PubMed: 18076762]

- Bhangoo SK, Ren D, Miller RJ, Chan DM, Ripsch MS, Weiss C, et al. CXCR4 chemokine receptor signaling mediates pain hypersensitivity in association with antiretroviral toxic neuropathy. *Brain Behav Immun* 2007b;21:581–591. [PubMed: 17292584]
- Bhangoo SK, Ripsch MS, Buchanan DJ, Miller RJ, White FA. Increased chemokine signaling in a model of HIV1-associated peripheral neuropathy. *Mol Pain* 2009;5:48. [PubMed: 19674450]
- Bogen O, Dina OA, Gear RW, Levine JD. Dependence of monocyte chemoattractant protein 1 induced hyperalgesia on the isolectin B4-binding protein versican. *Neuroscience* 2009;159:780–786. [PubMed: 19167466]
- Bonecchi R, Galliera E, Borroni EM, Corsi MM, Locati M, Mantovani A. Chemokines and chemokine receptors: an overview. *Front Biosci* 2009;14:540–551. [PubMed: 19273084]
- Bourd-Boittin K, Basset L, Bonnier D, L'Helgoualc'h A, Samson M, Theret N. CX3CL1/fractalkine shedding by human hepatic stellate cells: contribution to chronic inflammation in the liver. *J Cell Mol Med* 2009;13:1526–1535. [PubMed: 19432809]
- Buonamici S, Trimarchi T, Ruocco MG, Reavie L, Cathelin S, Mar BG, et al. CCR7 signalling as an essential regulator of CNS infiltration in T-cell leukaemia. *Nature* 2009;459:1000–1004. [PubMed: 19536265]
- Calderon TM, Eugenin EA, Lopez L, Kumar SS, Hesselgesser J, Raine CS, et al. A role for CXCL12 (SDF-1 α) in the pathogenesis of multiple sclerosis: regulation of CXCL12 expression in astrocytes by soluble myelin basic protein. *J Neuroimmunol* 2006;177:27–39. [PubMed: 16782208]
- Cartier L, Hartley O, Dubois-Dauphin M, Krause KH. Chemokine receptors in the central nervous system: role in brain inflammation and neurodegenerative diseases. *Brain Res Brain Res Rev* 2005;48:16–42. [PubMed: 15708626]
- Chapman GA, Moores K, Harrison D, Campbell CA, Stewart BR, Strijbos PJ. Fractalkine cleavage from neuronal membranes represents an acute event in the inflammatory response to excitotoxic brain damage. *J Neurosci* 2000;20:RC87. [PubMed: 10899174]
- Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med* 2006;354:610–621. [PubMed: 16467548]
- Clark AK, Gentry C, Bradbury EJ, McMahon SB, Malcangio M. Role of spinal microglia in rat models of peripheral nerve injury and inflammation. *Eur J Pain* 2007a;11:223–230. [PubMed: 16545974]
- Clark AK, Staniland AA, Marchand F, Kaan TKY, McMahon SB, Malcangio M. P2X7-dependent release of interleukin-1 β and nociception in the spinal cord following lipopolysaccharide. *J Neurosci* 2010;30:573–582. [PubMed: 20071520]
- Clark AK, Yip PK, Grist J, Gentry C, Staniland AA, Marchand F, et al. Inhibition of spinal microglial cathepsin S for the reversal of neuropathic pain. *Proc Natl Acad Sci U S A* 2007b;104:10655–10660. [PubMed: 17551020]
- Clark AK, Yip PK, Malcangio M. The liberation of fractalkine in the dorsal horn requires microglial cathepsin S. *J Neurosci* 2009a;29:6945–6954. [PubMed: 19474321]
- Clark, AK.; Wodarski, R.; Malcangio, M. Abstract Viewer/Itinerary Planner. Society for Neuroscience; Chicago: 2009b. CX3CR1 receptor is required for full development of nociceptive behavior following peripheral nerve injury. Online
- Costigan M, Moss A, Latremoliere A, Johnston C, Verma-Gandhu M, Herbert TA, et al. T-Cell Infiltration and Signaling in the Adult Dorsal Spinal Cord Is a Major Contributor to Neuropathic Pain-Like Hypersensitivity. *J Neurosci* 2009a;29:14415–14422. [PubMed: 19923276]
- Costigan M, Scholz J, Woolf CJ. Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu Rev Neurosci* 2009b;32:1–32. [PubMed: 19400724]
- Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, et al. BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 2005;438:1017–1021. [PubMed: 16355225]
- Croitoru-Lamourey J, Guillemin GJ, Boussin FD, Moggetti B, Gigout LI, Cheret A, et al. Expression of chemokines and their receptors in human and simian astrocytes: evidence for a central role of TNF α and IFN γ in CXCR4 and CCR5 modulation. *Glia* 2003;41:354–370. [PubMed: 12555203]

- Dansereau MA, Gosselin RD, Pohl M, Pommier B, Mechighel P, Mauborgne A, et al. Spinal CCL2 pronociceptive action is no longer effective in CCR2 receptor antagonist-treated rats. *J Neurochem* 2008;106:757–769. [PubMed: 18419759]
- Decosterd I, Woolf CJ. Spared nerve injury: an animal model of persistent peripheral neuropathic pain. *Pain* 2000;87:149–158. [PubMed: 10924808]
- DeLeo JA, Tanga FY, Tawfik VL. Neuroimmune activation and neuroinflammation in chronic pain and opioid tolerance/hyperalgesia. *Neuroscientist* 2004;10:40–52. [PubMed: 14987447]
- Devor M, Wall PD. Plasticity in the spinal cord sensory map following peripheral nerve injury in rats. *J Neurosci* 1981;1:679–684. [PubMed: 7346576]
- Dworkin RH, Backonja M, Rowbotham MC, Allen RR, Argoff CR, Bennett GJ, et al. Advances in neuropathic pain: diagnosis, mechanisms, and treatment recommendations. *Arch Neurol* 2003;60:1524–1534. [PubMed: 14623723]
- El-Hage N, Gurwell JA, Singh IN, Knapp PE, Nath A, Hauser KF. Synergistic increases in intracellular Ca²⁺, and the release of MCP-1, RANTES, and IL-6 by astrocytes treated with opiates and HIV-1 Tat. *Glia* 2005;50:91–106. [PubMed: 15630704]
- Fife BT, Huffnagle GB, Kuziel WA, Karpus WJ. CC chemokine receptor 2 is critical for induction of experimental autoimmune encephalomyelitis. *J Exp Med* 2000;192:899–905. [PubMed: 10993920]
- Filipovic R, Jakovcevski I, Zecevic N. GRO-alpha and CXCR2 in the human fetal brain and multiple sclerosis lesions. *Dev Neurosci* 2003;25:279–290. [PubMed: 12966224]
- Gao YJ, Ji RR. c-Fos and pERK, which is a better marker for neuronal activation and central sensitization after noxious stimulation and tissue injury? *Open Pain J* 2009;2:11–17. [PubMed: 19898681]
- Gao YJ, Zhang L, Samad OA, Suter MR, Yasuhiko K, Xu ZZ, et al. JNK-induced MCP-1 production in spinal cord astrocytes contributes to central sensitization and neuropathic pain. *J Neurosci* 2009;29:4096–4108. [PubMed: 19339605]
- Giovannelli A, Limatola C, Ragozzino D, Mileo AM, Ruggieri A, Ciotti MT, et al. CXC chemokines interleukin-8 (IL-8) and growth-related gene product alpha (GROalpha) modulate Purkinje neuron activity in mouse cerebellum. *J Neuroimmunol* 1998;92:122–132. [PubMed: 9916887]
- Glabinski AR, Balasingam V, Tani M, Kunkel SL, Strieter RM, Yong VW, et al. Chemokine monocyte chemoattractant protein-1 is expressed by astrocytes after mechanical injury to the brain. *J Immunol* 1996;156:4363–4368. [PubMed: 8666808]
- Gosselin RD, Varela C, Banisadr G, Mechighel P, Rostene W, Kitabgi P, et al. Constitutive expression of CCR2 chemokine receptor and inhibition by MCP-1/CCL2 of GABA-induced currents in spinal cord neurones. *J Neurochem* 2005;95:1023–1034. [PubMed: 16150057]
- Guo W, Wang H, Watanabe M, Shimizu K, Zou S, LaGraize SC, et al. Glial-cytokine-neuronal interactions underlying the mechanisms of persistent pain. *J Neurosci* 2007;27:6006–6018. [PubMed: 17537972]
- Harrison JK, Jiang Y, Chen S, Xia Y, Maciejewski D, McNamara RK, et al. Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. *Proc Natl Acad Sci U S A* 1998;95:10896–10901. [PubMed: 9724801]
- Holmes FE, Arnott N, Vanderplank P, Kerr NC, Longbrake EE, Popovich PG, et al. Intra-neural administration of fractalkine attenuates neuropathic pain-related behaviour. *J Neurochem* 2008;106:640–649. [PubMed: 18410510]
- Horuk R, Martin AW, Wang Z, Schweitzer L, Gerassimides A, Guo H, et al. Expression of chemokine receptors by subsets of neurons in the central nervous system. *J Immunol* 1997;158:2882–2890. [PubMed: 9058825]
- Hosoi R, Okada M, Hatazawa J, Gee A, Inoue O. Effect of astrocytic energy metabolism depressant on ¹⁴C-acetate uptake in intact rat brain. *J Cereb Blood Flow Metab* 2004;24:188–190. [PubMed: 14747745]
- Hu SJ, Xing JL. An experimental model for chronic compression of dorsal root ganglion produced by intervertebral foramen stenosis in the rat. *Pain* 1998;77:15–23. [PubMed: 9755014]
- Hua XY, Svensson CI, Matsui T, Fitzsimmons B, Yaksh TL, Webb M. Intrathecal minocycline attenuates peripheral inflammation-induced hyperalgesia by inhibiting p38 MAPK in spinal microglia. *Eur J Neurosci* 2005;22:2431–2440. [PubMed: 16307586]

- Inoue K. The function of microglia through purinergic receptors: neuropathic pain and cytokine release. *Pharmacol Ther* 2006;109:210–226. [PubMed: 16169595]
- Inoue K, Tsuda M. Microglia and neuropathic pain. *Glia* 2009;57:1469–1479. [PubMed: 19306358]
- Jeon SM, Lee KM, Cho HJ. Expression of monocyte chemoattractant protein-1 in rat dorsal root ganglia and spinal cord in experimental models of neuropathic pain. *Brain Res* 2009;1251:103–111. [PubMed: 19059387]
- Jeon SM, Lee KM, Park ES, Jeon YH, Cho HJ. Monocyte chemoattractant protein-1 immunoreactivity in sensory ganglia and hindpaw after adjuvant injection. *Neuroreport* 2008;19:183–186. [PubMed: 18185105]
- Ji RR, Baba H, Brenner GJ, Woolf CJ. Nociceptive-specific activation of ERK in spinal neurons contributes to pain hypersensitivity. *Nat Neurosci* 1999;2:1114–1119. [PubMed: 10570489]
- Ji RR, Kawasaki Y, Zhuang ZY, Wen YR, Decosterd I. Possible role of spinal astrocytes in maintaining chronic pain sensitization: review of current evidence with focus on bFGF/JNK pathway. *Neuron Glia Biology* 2006;2:259–269. [PubMed: 17710215]
- Ji RR, Kohno T, Moore KA, Woolf CJ. Central sensitization and LTP: do pain and memory share similar mechanisms? *Trends Neurosci* 2003;26:696–705. [PubMed: 14624855]
- Ji RR, Strichartz G. Cell signaling and the genesis of neuropathic pain. *Sci STKE* 2004;2004:reE14. [PubMed: 15454629]
- Ji RR, Suter MR. p38 MAPK, microglial signaling, and neuropathic pain. *Mol Pain* 2007;3:33. [PubMed: 17974036]
- Ji RR, Gereau RW 4th, Malcangio M, Strichartz GR. MAP kinase and pain. *Brain Res Rev* 2009a;60:135–48. [PubMed: 19150373]
- Ji RR, Xu ZZ, Wang X, Lo EH. Matrix metalloprotease regulation of neuropathic pain. *Trends Pharmacol Sci* 2009b;30:336–340. [PubMed: 19523695]
- Jin SX, Zhuang ZY, Woolf CJ, Ji RR. p38 mitogen-activated protein kinase is activated after a spinal nerve ligation in spinal cord microglia and dorsal root ganglion neurons and contributes to the generation of neuropathic pain. *J Neurosci* 2003;23:4017–4022. [PubMed: 12764087]
- Joseph EK, Chen X, Khasar SG, Levine JD. Novel mechanism of enhanced nociception in a model of AIDS therapy-induced painful peripheral neuropathy in the rat. *Pain* 2004;107:147–158. [PubMed: 14715401]
- Julius D, Basbaum AI. Molecular mechanisms of nociception. *Nature* 2001;413:203–210. [PubMed: 11557989]
- Jung H, Bhargoo S, Banisadr G, Freitag C, Ren D, White FA, et al. Visualization of chemokine receptor activation in transgenic mice reveals peripheral activation of CCR2 receptors in states of neuropathic pain. *J Neurosci* 2009;29:8051–8062. [PubMed: 19553445]
- Jung H, Toth PT, White FA, Miller RJ. Monocyte chemoattractant protein-1 functions as a neuromodulator in dorsal root ganglia neurons. *J Neurochem* 2008;104:254–263. [PubMed: 17944871]
- Karim F, Wang CC, Gereau RW 4th. Metabotropic glutamate receptor subtypes 1 and 5 are activators of extracellular signal-regulated kinase signaling required for inflammatory pain in mice. *J Neurosci* 2001;21:3771–3779. [PubMed: 11356865]
- Kawasaki Y, Xu ZZ, Wang X, Park JY, Zhuang ZY, Tan PH, et al. Distinct roles of matrix metalloproteases in the early- and late-phase development of neuropathic pain. *Nat Med* 2008a;14:331–336. [PubMed: 18264108]
- Kawasaki Y, Zhang L, Cheng JK, Ji RR. Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. *J Neurosci* 2008b;28:5189–5194. [PubMed: 18480275]
- Kehlet H, Jensen TS, Woolf CJ. Persistent postsurgical pain: risk factors and prevention. *Lancet* 2006;367:1618–1625. [PubMed: 16698416]
- Kim SH, Chung JM. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. *Pain* 1992;50:355–363. [PubMed: 1333581]

- Knerlich-Lukoschus F, Juraschek M, Blomer U, Lucius R, Mehdorn HM, Held-Feindt J. Force-dependent development of neuropathic central pain and time-related CCL2/CCR2 expression after graded spinal cord contusion injuries of the rat. *J Neurotrauma* 2008;25:427–448. [PubMed: 18338959]
- Kohn T, Ji RR, Ito N, Allchorne AJ, Befort K, Karchewski LA, et al. Peripheral axonal injury results in reduced mu opioid receptor pre- and post-synaptic action in the spinal cord. *Pain* 2005;117:77–87. [PubMed: 16098668]
- Kurihara T, Bravo R. Cloning and functional expression of mCCR2, a murine receptor for the C-C chemokines JE and FIC. *J Biol Chem* 1996;271:11603–11607. [PubMed: 8662823]
- Laing KJ, Secombes CJ. Chemokines. *Dev Comp Immunol* 2004;28:443–460. [PubMed: 15062643]
- Ledeboer A, Jekich BM, Sloane EM, Mahoney JH, Langer SJ, Milligan ED, et al. Intrathecal interleukin-10 gene therapy attenuates paclitaxel-induced mechanical allodynia and proinflammatory cytokine expression in dorsal root ganglia in rats. *Brain Behav Immun* 2007;21:686–698. [PubMed: 17174526]
- Ledeboer A, Sloane EM, Milligan ED, Frank MG, Mahony JH, Maier SF, et al. Minocycline attenuates mechanical allodynia and proinflammatory cytokine expression in rat models of pain facilitation. *Pain* 2005;115:71–83. [PubMed: 15836971]
- Lee HL, Lee KM, Son SJ, Hwang SH, Cho HJ. Temporal expression of cytokines and their receptors mRNAs in a neuropathic pain model. *Neuroreport* 2004;15:2807–2811. [PubMed: 15597059]
- Limatola C, Giovannelli A, Maggi L, Ragozzino D, Castellani L, Ciotti MT, et al. SDF-1 α -mediated modulation of synaptic transmission in rat cerebellum. *Eur J Neurosci* 2000;12:2497–2504. [PubMed: 10947825]
- Lindia JA, McGowan E, Jochnowitz N, Abbadi C. Induction of CX3CL1 expression in astrocytes and CX3CR1 in microglia in the spinal cord of a rat model of neuropathic pain. *J Pain* 2005;6:434–438. [PubMed: 15993821]
- Luster AD. Chemokines--chemotactic cytokines that mediate inflammation. *N Engl J Med* 1998;338:436–445. [PubMed: 9459648]
- McMahon SB, Malcangio M. Current challenges in glia-pain biology. *Neuron* 2009;64:46–54. [PubMed: 19840548]
- McManus C, Berman JW, Brett FM, Staunton H, Farrell M, Brosnan CF. MCP-1, MCP-2 and MCP-3 expression in multiple sclerosis lesions: an immunohistochemical and in situ hybridization study. *J Neuroimmunol* 1998;86:20–29. [PubMed: 9655469]
- Meeuwse S, Persoon-Deen C, Bsibsi M, Ravid R, van Noort JM. Cytokine, chemokine and growth factor gene profiling of cultured human astrocytes after exposure to proinflammatory stimuli. *Glia* 2003;43:243–253. [PubMed: 12898703]
- Menetski J, Mistry S, Lu M, Mudgett JS, Ransohoff RM, Demartino JA, et al. Mice overexpressing chemokine ligand 2 (CCL2) in astrocytes display enhanced nociceptive responses. *Neuroscience* 2007;149:706–714. [PubMed: 17870246]
- Mennicken F, Maki R, de Souza EB, Quirion R. Chemokines and chemokine receptors in the CNS: a possible role in neuroinflammation and patterning. *Trends Pharmacol Sci* 1999;20:73–78. [PubMed: 10101968]
- Miller RJ, Rostene W, Apartis E, Banisadr G, Biber K, Milligan ED, et al. Chemokine action in the nervous system. *J Neurosci* 2008;28:11792–11795. [PubMed: 19005041]
- Milligan E, Zapata V, Schoeniger D, Chacur M, Green P, Poole S, et al. An initial investigation of spinal mechanisms underlying pain enhancement induced by fractalkine, a neuronally released chemokine. *Eur J Neurosci* 2005;22:2775–2782. [PubMed: 16324111]
- Milligan ED, Sloane EM, Watkins LR. Glia in pathological pain: A role for fractalkine. *Journal of Neuroimmunology* 2008;198:113–120. [PubMed: 18547654]
- Milligan ED, Twining C, Chacur M, Biedenkapp J, O'Connor K, Poole S, et al. Spinal glia and proinflammatory cytokines mediate mirror-image neuropathic pain in rats. *J Neurosci* 2003;23:1026–1040. [PubMed: 12574433]
- Milligan ED, Watkins LR. Pathological and protective roles of glia in chronic pain. *Nat Rev Neurosci* 2009;10:23–36. [PubMed: 19096368]

- Milligan ED, Zapata V, Chacur M, Schoeniger D, Biedenkapp J, O'Connor KA, et al. Evidence that exogenous and endogenous fractalkine can induce spinal nociceptive facilitation in rats. *Eur J Neurosci* 2004;20:2294–2302. [PubMed: 15525271]
- Mines M, Ding Y, Fan GH. The many roles of chemokine receptors in neurodegenerative disorders: emerging new therapeutical strategies. *Curr Med Chem* 2007;14:2456–2470. [PubMed: 17979699]
- Moalem G, Tracey DJ. Immune and inflammatory mechanisms in neuropathic pain. *Brain Res Rev* 2006;51:240–264. [PubMed: 16388853]
- Mojsilovic-Petrovic J, Callaghan D, Cui H, Dean C, Stanimirovic DB, Zhang W. Hypoxia-inducible factor-1 (HIF-1) is involved in the regulation of hypoxia-stimulated expression of monocyte chemoattractant protein-1 (MCP-1/CCL2) and MCP-5 (Ccl12) in astrocytes. *J Neuroinflammation* 2007;4:12. [PubMed: 17474992]
- Murphy CA, Hoek RM, Wiekowski MT, Lira SA, Sedgwick JD. Interactions between hemopoietically derived TNF and central nervous system-resident glial chemokines underlie initiation of autoimmune inflammation in the brain. *J Immunol* 2002;169:7054–7062. [PubMed: 12471141]
- Nishiyori A, Minami M, Ohtani Y, Takami S, Yamamoto J, Kawaguchi N, et al. Localization of fractalkine and CX3CR1 mRNAs in rat brain: does fractalkine play a role in signaling from neuron to microglia? *FEBS Lett* 1998;429:167–172. [PubMed: 9650583]
- Oh SB, Tran PB, Gillard SE, Hurley RW, Hammond DL, Miller RJ. Chemokines and glycoprotein120 produce pain hypersensitivity by directly exciting primary nociceptive neurons. *J Neurosci* 2001;21:5027–5035. [PubMed: 11438578]
- Ohtori S, Takahashi K, Moriya H, Myers RR. TNF-alpha and TNF-alpha receptor type 1 upregulation in glia and neurons after peripheral nerve injury: studies in murine DRG and spinal cord. *Spine* 2004;29:1082–1088. [PubMed: 15131433]
- Pan Y, Lloyd C, Zhou H, Dolich S, Deeds J, Gonzalo JA, et al. Neurotactin, a membrane-anchored chemokine upregulated in brain inflammation. *Nature* 1997;387:611–617. [PubMed: 9177350]
- Pease JE, Horuk R. Chemokine receptor antagonists: Part 1. *Expert Opin Ther Pat* 2009a;19:39–58. [PubMed: 19441897]
- Pease JE, Horuk R. Chemokine receptor antagonists: part 2. *Expert Opin Ther Pat* 2009b;19:199–221. [PubMed: 19441918]
- Polomano RC, Mannes AJ, Clark US, Bennett GJ. A painful peripheral neuropathy in the rat produced by the chemotherapeutic drug, paclitaxel. *Pain* 2001;94:293–304. [PubMed: 11731066]
- Porreca F, Ossipov MH, Gebhart GF. Chronic pain and medullary descending facilitation. *Trends Neurosci* 2002;25:319–325. [PubMed: 12086751]
- Raghavendra V, Tanga F, DeLeo JA. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. *J Pharmacol Exp Ther* 2003;306:624–630. [PubMed: 12734393]
- Romero-Sandoval EA, Horvath RJ, DeLeo JA. Neuroimmune interactions and pain: focus on glial-modulating targets. *Curr Opin Investig Drugs* 2008;9:726–734.
- Romero-Sandoval EA, Horvath R, Landry RP, DeLeo JA. Cannabinoid receptor type 2 activation induces a microglial anti-inflammatory phenotype and reduces migration via MKP induction and ERK dephosphorylation. *Mol Pain* 2009;5:25. [PubMed: 19476641]
- Rossi D, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol* 2000;18:217–242. [PubMed: 10837058]
- Rottman JB, Slavin AJ, Silva R, Weiner HL, Gerard CG, Hancock WW. Leukocyte recruitment during onset of experimental allergic encephalomyelitis is CCR1 dependent. *Eur J Immunol* 2000;30:2372–2377. [PubMed: 10940928]
- Savarin-Vuillat C, Ransohoff RM. Chemokines and chemokine receptors in neurological disease: raise, retain, or reduce? *Neurotherapeutics* 2007;4:590–601. [PubMed: 17920540]
- Schafers M, Lee DH, Brors D, Yaksh TL, Sorkin LS. Increased sensitivity of injured and adjacent uninjured rat primary sensory neurons to exogenous tumor necrosis factor-alpha after spinal nerve ligation. *J Neurosci* 2003;23:3028–3038. [PubMed: 12684490]
- Scholz J, Woolf CJ. Can we conquer pain? *Nat Neurosci* 2002;5(Suppl):1062–1067. [PubMed: 12403987]

- Scholz J, Woolf CJ. The neuropathic pain triad: neurons, immune cells and glia. *Nat Neurosci* 2007;10:1361–1368. [PubMed: 17965656]
- Seltzer Z, Dubner R, Shir Y. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. *Pain* 1990;43:205–218. [PubMed: 1982347]
- Simpson J, Rezaie P, Newcombe J, Cuzner ML, Male D, Woodroffe MN. Expression of the beta-chemokine receptors CCR2, CCR3 and CCR5 in multiple sclerosis central nervous system tissue. *J Neuroimmunol* 2000a;108:192–200. [PubMed: 10900353]
- Simpson JE, Newcombe J, Cuzner ML, Woodroffe MN. Expression of monocyte chemoattractant protein-1 and other beta-chemokines by resident glia and inflammatory cells in multiple sclerosis lesions. *J Neuroimmunol* 1998;84:238–249. [PubMed: 9628469]
- Simpson JE, Newcombe J, Cuzner ML, Woodroffe MN. Expression of the interferon-gamma-inducible chemokines IP-10 and Mig and their receptor, CXCR3, in multiple sclerosis lesions. *Neuropathol Appl Neurobiol* 2000b;26:133–142. [PubMed: 10840276]
- Sommer C, Kress M. Recent findings on how proinflammatory cytokines cause pain: peripheral mechanisms in inflammatory and neuropathic hyperalgesia. *Neurosci Lett* 2004;361:184–187. [PubMed: 15135924]
- Sun JH, Yang B, Donnelly DF, Ma C, LaMotte RH. MCP-1 enhances excitability of nociceptive neurons in chronically compressed dorsal root ganglia. *J Neurophysiol* 2006;96:2189–2199. [PubMed: 16775210]
- Tanaka T, Minami M, Nakagawa T, Satoh M. Enhanced production of monocyte chemoattractant protein-1 in the dorsal root ganglia in a rat model of neuropathic pain: possible involvement in the development of neuropathic pain. *Neurosci Res* 2004;48:463–469. [PubMed: 15041200]
- Tanuma N, Sakuma H, Sasaki A, Matsumoto Y. Chemokine expression by astrocytes plays a role in microglia/macrophage activation and subsequent neurodegeneration in secondary progressive multiple sclerosis. *Acta Neuropathol* 2006;112:195–204. [PubMed: 16733654]
- Tawfik VL, Natile-McMenemy N, Lacroix-Fralish ML, Deleo JA. Efficacy of propentofylline, a glial modulating agent, on existing mechanical allodynia following peripheral nerve injury. *Brain Behav Immun* 2007;21:238–246. [PubMed: 16949251]
- Thacker MA, Clark AK, Bishop T, Grist J, Yip PK, Moon LD, et al. CCL2 is a key mediator of microglia activation in neuropathic pain states. *Eur J Pain* 2009;13:263–272. [PubMed: 18554968]
- Trang T, Beggs S, Wan X, Salter MW. P2X4-receptor-mediated synthesis and release of brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated protein kinase activation. *J Neurosci* 2009;29:3518–3528. [PubMed: 19295157]
- Trebst C, Staugaitis SM, Tucky B, Wei T, Suzuki K, Aldape KD, et al. Chemokine receptors on infiltrating leucocytes in inflammatory pathologies of the central nervous system (CNS). *Neuropathol Appl Neurobiol* 2003;29:584–595. [PubMed: 14636165]
- Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, et al. P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* 2003;424:778–83. [PubMed: 12917686]
- Tsuda M, Mizokoshi A, Shigemoto-Mogami Y, Koizumi S, Inoue K. Activation of p38 mitogen-activated protein kinase in spinal hyperactive microglia contributes to pain hypersensitivity following peripheral nerve injury. *Glia* 2004;45:89–95. [PubMed: 14648549]
- Tuppo EE, Arias HR. The role of inflammation in Alzheimer's disease. *Int J Biochem Cell Biol* 2005;37:289–305. [PubMed: 15474976]
- Ubogu EE, Cossoy MB, Ransohoff RM. The expression and function of chemokines involved in CNS inflammation. *Trends Pharmacol Sci* 2006;27:48–55. [PubMed: 16310865]
- Van Der Voorn P, Tekstra J, Beelen RH, Tensen CP, Van Der Valk P, De Groot CJ. Expression of MCP-1 by reactive astrocytes in demyelinating multiple sclerosis lesions. *Am J Pathol* 1999;154:45–51. [PubMed: 9916917]
- Verge GM, Milligan ED, Maier SF, Watkins LR, Naeve GS, Foster AC. Fractalkine (CX3CL1) and fractalkine receptor (CX3CR1) distribution in spinal cord and dorsal root ganglia under basal and neuropathic pain conditions. *Eur J Neurosci* 2004;20:1150–1160. [PubMed: 15341587]

- Wallace VC, Cottrell DF, Brophy PJ, Fleetwood-Walker SM. Focal lysolecithin-induced demyelination of peripheral afferents results in neuropathic pain behavior that is attenuated by cannabinoids. *J Neurosci* 2003;23:3221–3233. [PubMed: 12716929]
- Watkins LR, Maier SF. Beyond neurons: evidence that immune and glial cells contribute to pathological pain states. *Physiol Rev* 2002;82:981–1011. [PubMed: 12270950]
- Watkins LR, Maier SF. Glia: a novel drug discovery target for clinical pain. *Nat Rev Drug Discov* 2003;2:973–985. [PubMed: 14654796]
- Weeraratna AT, Kalehua A, Deleon I, Bertak D, Maher G, Wade MS, et al. Alterations in immunological and neurological gene expression patterns in Alzheimer's disease tissues. *Exp Cell Res* 2007;313:450–461. [PubMed: 17188679]
- White FA, Bhargoo SK, Miller RJ. Chemokines: integrators of pain and inflammation. *Nat Rev Drug Discov* 2005a;4:834–844. [PubMed: 16224455]
- White FA, Jung H, Miller RJ. Chemokines and the pathophysiology of neuropathic pain. *Proc Natl Acad Sci U S A* 2007;104:20151–20158. [PubMed: 18083844]
- White FA, Sun J, Waters SM, Ma C, Ren D, Ripsch M, et al. Excitatory monocyte chemoattractant protein-1 signaling is up-regulated in sensory neurons after chronic compression of the dorsal root ganglion. *Proc Natl Acad Sci U S A* 2005b;102:14092–14097. [PubMed: 16174730]
- White FA, Wilson NM. Chemokines as pain mediators and modulators. *Current Opinion in Anesthesiology* 2008;21:580–585. [PubMed: 18784482]
- Woolf CJ, Mannion RJ. Neuropathic pain: aetiology, symptoms, mechanisms, and management. *Lancet* 1999;353:1959–1964. [PubMed: 10371588]
- Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. *Science* 2000;288:1765–1769. [PubMed: 10846153]
- Xia M, Hyman BT. GROalpha/KC, a chemokine receptor CXCR2 ligand, can be a potent trigger for neuronal ERK1/2 and PI-3 kinase pathways and for tau hyperphosphorylation-a role in Alzheimer's disease? *J Neuroimmunol* 2002;122:55–64. [PubMed: 11777543]
- Xia M, Qin S, McNamara M, Mackay C, Hyman BT. Interleukin-8 receptor B immunoreactivity in brain and neuritic plaques of Alzheimer's disease. *Am J Pathol* 1997;150:1267–1274. [PubMed: 9094983]
- Xia MQ, Bacskaï BJ, Knowles RB, Qin SX, Hyman BT. Expression of the chemokine receptor CXCR3 on neurons and the elevated expression of its ligand IP-10 in reactive astrocytes: in vitro ERK1/2 activation and role in Alzheimer's disease. *J Neuroimmunol* 2000;108:227–235. [PubMed: 10900358]
- Xia MQ, Qin SX, Wu LJ, Mackay CR, Hyman BT. Immunohistochemical study of the beta-chemokine receptors CCR3 and CCR5 and their ligands in normal and Alzheimer's disease brains. *Am J Pathol* 1998;153:31–37. [PubMed: 9665462]
- Yan YP, Sailor KA, Lang BT, Park SW, Vemuganti R, Dempsey RJ. Monocyte chemoattractant protein-1 plays a critical role in neuroblast migration after focal cerebral ischemia. *J Cereb Blood Flow Metab* 2007;27:1213–1224. [PubMed: 17191078]
- Yang EK, Takimoto K, Hayashi Y, de Groat WC, Yoshimura N. Altered expression of potassium channel subunit mRNA and alpha-dendrotoxin sensitivity of potassium currents in rat dorsal root ganglion neurons after axotomy. *Neuroscience* 2004;123:867–874. [PubMed: 14751280]
- Zhang J, De Koninck Y. Spatial and temporal relationship between monocyte chemoattractant protein-1 expression and spinal glial activation following peripheral nerve injury. *J Neurochem* 2006;97:772–783. [PubMed: 16524371]
- Zhang J, Shi XQ, Echeverry S, Mogil JS, De Koninck Y, Rivest S. Expression of CCR2 in both resident and bone marrow-derived microglia plays a critical role in neuropathic pain. *J Neurosci* 2007;27:12396–12406. [PubMed: 17989304]
- Zhao P, Waxman SG, Hains BC. Modulation of thalamic nociceptive processing after spinal cord injury through remote activation of thalamic microglia by cysteine cysteine chemokine ligand 21. *J Neurosci* 2007;27:8893–8902. [PubMed: 17699671]
- Zhuang ZY, Kawasaki Y, Tan PH, Wen YR, Huang J, Ji RR. Role of the CX3CR1/p38 MAPK pathway in spinal microglia for the development of neuropathic pain following nerve injury-induced cleavage of fractalkine. *Brain Behav Immun* 2007;21:642–651. [PubMed: 17174525]

- Zhuang ZY, Wen YR, Zhang DR, Borsello T, Bonny C, Strichartz GR, et al. A peptide c-Jun N-terminal kinase (JNK) inhibitor blocks mechanical allodynia after spinal nerve ligation: respective roles of JNK activation in primary sensory neurons and spinal astrocytes for neuropathic pain development and maintenance. *J Neurosci* 2006;26:3551–3560. [PubMed: 16571763]
- Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. *Immunity* 2000;12:121–127. [PubMed: 10714678]

Figure 1. Spinal nerve ligation (SNL) induces a substantial upregulation of CX3CR1 in spinal cord microglial cells

(a) Upregulation of CX3CR1 in the ipsilateral side of the spinal cord (L5), 3 days after ligation of the L5 spinal nerve. (b–d) Double staining reveals a complete co-localization of CX3CR1 with the microglial marker OX-42 in the dorsal horn. Scales, 100 μ m. The images are reproduced from a paper by Zhuang et al., 2007 (*Brain, Behavior, and Immunity*) with permission.

Figure 2. Neuronal-microglial interactions: schematic showing how chemokines (CX3CL1, CCL2) produced from primary sensory neurons after nerve injury activate microglia in the spinal cord for the generation of neuropathic pain

Spinal microglial cells express the chemokine receptors CX3CR1 and CCR2. Activation of these receptors induces p38 phosphorylation in microglia, leading to the production of proinflammatory cytokines and growth factor, and subsequent sensitization of dorsal horn neurons (central sensitization) and neuropathic pain facilitation. Nerve injury also produces the protease cathepsin S (CatS) from microglia, which can cleave CX3CL1 from the cell surface of neurons (primary sensory and dorsal horn neurons) and astrocytes, leading to further activation of microglia.

Figure 3. Spinal nerve ligation (SNL) induces upregulation of CCL2 in spinal cord astrocytes
(a, b) Upregulation of CCL2 in the ipsilateral side of the spinal cord, 3 days after ligation of the spinal nerve. **(c)** Double staining reveals a co-localization of CCL2 (red) with astrocyte marker GFAP (green) in the superficial dorsal horn. **(d)** Double staining of CCR2 (green) with neuronal marker NeuN (red) in the dorsal horn of naïve animals. Spinal cords were obtained from CCR2-GFP mice. Scales, 50 μ m. The images are re-produced from a paper by Gao et al., 2009 (*J Neurosci*) with permission.

Figure 4. Perfusion of spinal cord slices with CCL2 (100 ng/ml) enhances NMDA-induced current (a) and induce rapid phosphorylation of ERK (pERK) in superficial dorsal horn neurons (b) Five minutes after CCL2 stimulation, the spinal cord slices were fixed and processed for pERK immunostaining. Scale, 100 μ m. The data are re-produced from a paper by Gao et al., 2009 (*J Neurosci*) with permission.

Figure 5. Astroglial-neuronal interactions: schematic showing how chemokine (CCL2) produced from spinal cord astrocytes directly activates dorsal horn neurons in the spinal cord for the generation of neuropathic pain

Nerve injury produces TNF- α from microglia, which activates JNK in astrocytes. JNK activation in turn produces CCL2 in astrocytes. Activation of CCR2 in spinal cord neurons by CCL2 produces central sensitization via ERK-mediated activation of NMDA receptors, leading to an enhanced neuropathic pain states.

Figure 6. Strategies of targeting chemokine signaling for reducing neuropathic pain

In addition to direct targeting the chemokines with neutralizing antibodies and the chemokine receptors with antagonists, indirect targeting the synthesis of chemokines with inhibitors of NF- κ B and MAPKs and cleavage of chemokines with proteases inhibitors may offer new opportunities for improving efficacy.